# Clone-Forming Activity of Embryonal Stem Hemopoietic Cells after Transplantation to Newborn or Adult Sublethally Irradiated Mice

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Hemopoietic activity of stem hemopoietic cells from the liver of embryos was studied at different terms of intrauterine development. The fate of individual clones of hemopoietic cells marked by human adenosine deaminase gene was followed up in sublethally irradiated or newborn recipients. The efficiency of marker gene incorporation in primitive stem hemopoietic cells from the liver of 12-, 13-, and 17-day embryos was not high. Gene transfer was performed without cell prestimulation to division, and hence, these data show that primitive stem cells proliferate even in 17-day embryos. Cells from embryonal liver in all terms maintain hemopoiesis both in newborn and adult microenvironment, hemopoiesis being realized according to the clonal succession model, *i. e.* in the some way after transplantation of the bone marrow from adult mice.

**Key Words:** *primitive stem hemopoietic cell; embryonal liver; retrovirus gene transfer; splenic colony-forming unit; clonal succession*

Hemopoiesis is maintained by hemopoietic stem cells (HSC) characterized by high proliferative potential and capable of differentiating into different blood cells [8]. Normally HSC are in the state of deep rest and are mobilized to proliferation and differentiation successively forming clones replacing each other [3]. This process was denoted as clonal succession [6]. Experimental validation of clonal succession in the hemopoietic system was obtained in studies with HSC marked by retroviral gene transfer [5]. In adult animals hemopoiesis is maintained by many hemopoietic clones, HSC derivatives, functioning simultaneously.

Our purpose was to identify the stage of ontogeny when HSC acquire capacity to create hemopoiesis normally functioning in accordance with the clonal succession model and the role of the type of hemopoietic microenvironment in this process. In this study we traced the fate of HSC from the liver of 12-, 13- and 17-day mouse embryos labeled with human adenosine deaminase gene cDNA after their transplantation to adult sublethally irradiated mice or newborn mice conditioned with bisulfane *in utero.*

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## MATERIALS AND METHODS

Experiments were carried out on 12-17-day embryos of (C57Bl/6×CBA2)F1 mice. The day of detection of vaginal plug was taken as day 0 of pregnancy. The liver of 12-, 13-, and 17-day embryos was crushed by passing through a syringe with a needle of caliber 18. The suspension of liver cells in α-MEM with 20% fetal calf serum was transferred into fibroblasts carrying a retrovirus containing human adenosine deaminase gene (hADA) and cDNA (hADA $GP^+E86$) preirradiated in a dose of 50 Gy [2]. After 48-h incubation in the producer strain, embryonal liver cells were injected intravenously to either sublethally irradiated (6 Gy) adult (12-16 weeks) CBF1 mice or intrahepatically to CBF1 mice during the first 18 h after birth

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(Table 1). The females raising their progeny were injected with bisulfane in a dose of 15 mg/kg in 1% methylcellulose (2 mg/ml) on days 17 and 18 of pregnancy [9]. Two and six months after transplantation bone marrow (BM) was obtained from recipients of both groups by puncture of the right and left femur in succession under light ether narcosis. BM cells from primary recipients were injected to second irradiated recipients (12 Gy, two fractions at 3-h interval, IPK $^{137}Cs$ device), and after 10 days individual splenic colonies were analyzed by PCR for hADA sequence in the DNA [5]. DNA from hADA-positive colonies was then analyzed by Southern blot hybridization for detecting unique hADA integration sites. The results were statistically processed using Student's *t* test.

**TABLE 1.** Dose of Injected Embryonal Liver Cells

| Embryonal age, days | Number of injected cells/mouse, $\times 10^6$ | Liver equivalent per mouse |
|---|---|---|
| 12 (*n*=35) | 4.5 | 1 |
| 13 (*n*=14) | 11.4 | 0.5 |
| 17 (*n*=7) | 7.1 | 0.4 |

## RESULTS

The level of restored hemopoiesis 2 and 6 months after injection of embryonal liver cells was practically the same in both groups of recipients. The concentration of splenic colony-forming units (CFUs) in BM was 8-13 per $2\times10^5$ cells irrespective of the age of embryonal liver and period of investigation (Table 2). It was found that the efficiency of incorporation of marker hADA gene in embryonal CFUs was 60-85% without prestimulation, as they actively proliferate [4]. The efficiency of marker incorporation in embryonal prestimulated HSC was not studied. In our experiment, gene incorporation (possible only in proliferating cells) in embryonal HSC was observed with embryonal liver cells of all ages (Table 2). Labeled CFUs were detected in both groups of recipients during 6 months after reconstitution, the number of labeled colonies did not virtually depend on the age of cells. The lifespan of CFUs in mice exceeded 1 month [1,7], and therefore, labeled colonies originate from HSC which incorporated a transferred foreign gene, while liver HSC in embryogenesis proliferate throughout the last third of pregnancy and the percentage of proliferating HSC does not decrease from the beginning of organogenesis (day 11) until birth (days 18-19). On the other hand, after birth more than 95% HSC are in the state of deep rest and do not incorporate provirus without potent prestimulation with hemopoietic growth factors. It seems that transition into a silent state depends not only on the age, but also on the site of hemopoiesis (migration of HSC from embryonal liver into BM) as well.

The efficiency of marker incorporation into HSC was very low. The percentage of labeled colonies in sublethally irradiated mice did not exceed 20% (Table 2). After injection of the same suspension to newborn mice the percentage of labeled colonies was even lower (Table 2). Hence, in contrast to younger (days 9-11) yolk sac, aortic, gonadal, and mesonephros HSC, repopulating the hemopoietic microenvironment of a newborn but not adult organism [9], HSC of 12-17-day-old embryonal liver need no early postnatal microenvironment and repopulate the hemopoietic system of the adult recipient at least not worse than in newborn.

We detected 125 marked CFUs presenting 54 clones in 45 experimental animals. Clonal kinetics in reconstituted animals cannot be studied in detail because of low repopulating activity of embryonal HSC. However hemopoiesis in the representative mice was polyclonal, and when labeled colonies were detected in both terms of investigation, the functioning clones were different (Table 3), which agrees with clonal succession detected in adult BM. In general, our findings indicate that HSC from embryonal liver marked under maximally gentle conditions possess the main characteristics of adult HSC even without prestimulation with exogenous cytokines: they need no early postembryonal microenvironment, transit into a state of deep rest after transplantation as it normally occurs, and are

**TABLE 2.** Content of CFUs in Mouse BM per $2\times10^5$ cells, (Percentage of Labeled CFUs in Parentheses, *M*±*m*)

| Age of embryonal liver cells, days | Time after reconstitution, months | | | |
|---|---|---|---|---|
| | 2 | | 6 | |
| | adult | newborns | adult | newborns |
| 12 | 7.6±1.8 (6.0±2.5) | 13.6±1.1 (3.0±2.8) | 8.9±0.8 (4.0±2.7) | 9.7±1.0 (0±0) |
| 13 | 9.7±0.6 (21.3±7.9) | 12.5±0.8 (5.0±1.8) | 8.7±0.7 (2.8±2.6) | 9.6±0.6 (2±1) |
| 17 | 11.4±0.6 (7.4±3.0) | 12.7±1.4 (0±0) | 10.7±1.2 (7.4±4.0) | 8.4±0.8 (3.5±2.3) |

**TABLE 3.** Distribution of Labeled CFUs Clones

| Recipients, mouse No. (age of injected liver cells, days) | | Time after reconstitution, months | |
|---|---|---|---|
| | | 2 | 6 |
| | | No. of CFUs clone | No. of CFUs clone |
| Irradiated | 6 (12) | 2 (1), 3 (1), 4 (1), 5 (2) | 6 (1) |
| | 10 (13) | 1 (1), 2 (1), 3 (1), 4 (2), 5 (1), 6 (1) | 7 (1)*, 8 (1), 9 (1) |
| | 16 (17) | 1 (1), 2 (1) | 3 (1)*, 4 (1), 5 (2) |
| | 18 (17) | 1 (1) | 2 (1), 3 (1) |
| | 20 (17) | 2 (1) | 3 (1)*, 4 (2), 5 (1), 6 (1) |
| | 21 (17) | 1 (1), 2 (1), 3 (1) | 4 (1) |
| Newborn | 26 (12) | 1 (1), 2 (3), 3 (1) | — |
| | 32 (13) | 1 (1), 2 (1) | 3 (1) |
| | 41 (17) | — | 1 (1), 2 (2), 3 (1) |

**Note.** Number of colonies presenting this clone is shown in parentheses. *The same clone was detected in BM.

mobilized into clone formation in succession in accordance with the clonal succession model.

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## REFERENCES

1. N. I. Drize, T. V. Todriya, and I. L. Chertkov, *Byull. Eksp. Biol. Med.*, **127**, No. 4, 419-423 (1999).
2. I. L. Chertkov and N. I. Drize, *Probl. Gematol.*, **2**, 19-29 (1996).
3. J. L. Abkowitz, M. L. Linenberger, M. A. Newton, *et al.*, *Proc. Natl. Acad. Sci. USA*, **87**, 9062-9066 (1990).
4. J. L. Chertkov, S. Jiang, J. D. Lutton, *et al.*, *Stem Cells*, **11**, 218-224 (1993).
5. N. I. Drize, D. Keller, and J. L. Chertkov, *Blood*, **88**, 2927-2938 (1996).
6. H. E. M. Kay, *Lancet*, **11**, 418-419 (1965).
7. G. Keller and R. Snodgrass, *J. Exp. Med.*, **171**, 1407-1416 (1990).
8. J. E. Till and E. A. McCulloch, *Biochim. Biophys. Acta*, **26**, 431-459 (1980).
9. M. C. Yoder, K. Hiatt, P. Dutt, *et al.*, *Immunity*, **7**, 335-344 (1997).